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DEVELOPMENT AND TESTING OF AN IN VITRO SAMPLE HOLDER FOR RADIOF--ETC(U)
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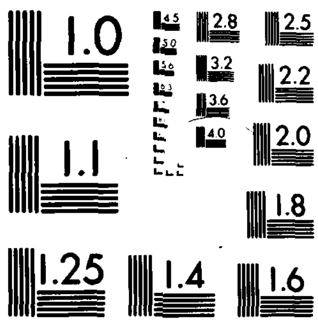

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DEVELOPMENT AND TESTING OF AN IN VITRO SAMPLE HOLDER FOR RADIOFREQUENCY RADIATION BIOEFFECTS RESEARCH

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Interim Report for Period March 1979 - March 1980

Approved for public release; distribution unlimited.

USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
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This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

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The results of an investigation into the design of a simple, low-cost device which would expose cell cultures and cell-free suspensions in vitro to known, uniform, and reproducible levels of radiofrequency radiation (RFR) are presented. The design selected consists of a linear array of 5 small cellulose nitrate centrifuge tubes with array oriented parallel to the E-field and the long axis of the tubes oriented parallel with the H-field. Reproducible uniform specific absorption rate (SAR) distributions were found through the frequency range of 0.48 to 10 GHz with SAR dependent on frequency.

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DEVELOPMENT AND TESTING OF AN IN VITRO SAMPLE HOLDER FOR RADIOFREQUENCY RADIATION BIOEFFECTS RESEARCH

INTRODUCTION

Radiofrequency radiation (RFR) bioeffects research has advanced to the stage where detailed investigations into cellular and subcellular effects must be made in an attempt to determine the mechanisms for possible RFR damage. To accomplish these studies, a device is needed that will allow the in vitro exposure of cell suspensions or cell-free suspensions (enzymes, RNA, DNA, etc.) to known, uniform, and reproducible quantities of RFR energy. Several in vitro exposure devices have been developed by other investigators, i.e., waveguide exposure system (1), transmission line termination exposure system (2), fluid-filled waveguide system (3), and various devices placed in the field of a radiating RF horn or antenna (4). Most of these devices have been dedicated systems with substantial support equipment required, were restricted in frequency range of exposure, and were poorly documented in the actual dose delivered to the biological sample.

We present in this Technical Report the results of an investigation into the design and testing of a simple, low-cost device which would expose cultures and cell-free suspensions in vitro to known, uniform, and reproducible levels of RFR energy.

MATERIALS AND METHODS

Introduction

The exposure device selected for testing in this study was a standard "test tube" supported in a block of Styrofoam HD300 (Dow Chemical Company, Midland MI). This type of device was selected due to its low cost, disposability, sterilizability, availability in various sizes and materials, small size with respect to RFR exposure equipment available, and sufficient volume of sample for potential biochemical and biophysical analyses. This device could be used in anechoic chambers in the far field and in transverse electric mode (TEM) chambers with equal ease, providing a wide range of exposure parameters. The Styrofoam block would provide necessary support for the test tubes, help maintain thermal equilibrium before and after exposures, allow the exposure of an array of tubes with a fixed geometric relationship, and not perturb the RFR field to any appreciable extent. The remaining questions that must be answered concerning the appropriateness of this device include: What is the energy distribution in the test tube under various exposure orientations? What effect does the size and type of test-tube material have? Are all test tubes in an array exposed to identical energy levels? How critical is the alignment of the exposure device in the field? Are the exposures reproducible? These questions were answered using the RFR exposure facilities and specific experiments listed below.

RFR Exposure Facilities

All RFR exposures were conducted in the USAF School of Aerospace Medicine (USAFSAM) anechoic chambers or Narda Model 8801 TEM cell, building 1187, using

the exposure parameters in Table 1. Radiofrequency power was brought to operating level as rapidly as possible (<2 sec) and monitored continuously using a Hewlett Packard HP 432 Power Meter. Chamber temperature was maintained at 23°C ±2°C for all exposures. Incident power densities were measured using a Narda Microwave Corp, Model 8316, Broad Band Isotropic RF Monitor with Model 8323 Probe.

TABLE 1. RFR EXPOSURE PARAMETERS

Frequency (GHz)	Power density (mW/cm^2)	Distance from horn (m)	Exposure device
0.48	450	N/A	Narda Model 8801 TEM Transmission Cell
1.20	70	0.8	RANTEC Anechoic Chamber Cober Model 1326 Transmitter
4.50	100	0.9	
10.00	50	0.9	

Specific Absorption Rate (SAR) Measurements

The SAR distributions in the in vitro sample holders were determined using initial rate of temperature rise data measured at specific locations in the test tubes. The initial rate of temperature rise may be used to estimate SAR in W/kg by knowing the specific heat of the material and several conversion factors.

$$\text{SAR(W/kg)} = \text{Initial rate of temp rise } \left(\frac{°C}{\text{sec}}\right) \times \text{ specific heat of material } \left(\frac{\text{Cal}}{\text{g-°C}}\right) \times \text{ conversion of g to kg } \left(\frac{1000 \text{ g}}{\text{kg}}\right) \times \text{ conversion of } \frac{\text{cal}}{\text{sec}} \text{ to watts } \left(\frac{4.186 \text{ watts}}{\text{cal/sec}}\right) = \text{rate of temp rise } \left(\frac{°C}{\text{sec}}\right) \times \text{ specific heat } \left(\frac{\text{cal}}{\text{g-°C}}\right) \times 4186$$

For the muscle equivalent material used in these experiments the specific heat was 0.84 cal/g-°C, therefore:

$$\text{SAR(W/kg)} = 3516.2 \times \text{temperature rise in } \frac{°C}{\text{sec}}$$

The initial rate of temperature rise was measured using a Vitek, Inc., Model 101, Electrothermia Monitor with output to a Data Precision, Model 3500,

Digital Multimeter, Microwave Labs ML 1200 Scanner and Hewlett Packard HP 9830A Computing Calculator. The Electrothermia Monitor (5) has a probe diameter of 1 mm, absolute accuracy better than $\pm 0.05°C$, stability better than $\pm 0.01°C$, time constant of 0.2 sec, and an RF line heating error of 0.005°C for an RF heating equivalent of 1°C/min. The Electrothermia Monitor was calibrated against a Thermometrics, Inc., S-10, Four Wire Thermistor Standard, Serial No. 282, which is traceable to the National Bureau of Standards (NBS) and had a reported uncertainty of less than 0.0015°C. The rate of rise in temperature was estimated by exposing the test tubes for 20 sec at incident power densities of between 50 and 70 mW/cm^2. Temperature rises during this short period were found to be quite linear due to the lack of time necessary for other heat transfer mechanisms to remove the absorbed heat from the sample point. Because of the variable incident power densities used, all results are reported in normalized SAR of W/kg per mW/cm^2 of incident power density. This method of reporting allows direct comparability of all experimental data.

Experimental Design

Various sizes of Styrofoam blocks were constructed to act as supports for test tubes of various size and material of construction so that exposures could be made in the far field of the RFR horn or TEM chamber. Test tubes were filled with muscle-equivalent material which consisted of 8.45% (by weight) of TX 150 (trade name for silicone gelling agent from Whamo Manufacturing Company), 15.2% Polyethylene Powder (Wedco California, Inc.), 0.9069% NaCl, and 75.44% H_2O. This muscle-equivalent material was developed by Guy et al. (6).

The following exposures were performed in an attempt to determine the optimum design of the multi-sample in vitro exposure device.

1. The effect of exposure orientation was determined using the Styrofoam test-tube holder shown in Figure 1 with the large (2.5 cm diameter x 6.6 cm long) and the small (1.3 cm diameter x 5.1 cm long) cellulose nitrate (CN) centrifuge tubes filled with muscle-equivalent material. Exposures were made with the long dimension of the tube parallel with the E, H, and K vectors, and the SAR was measured along the central axis, the front (K_1), rear (K_2), and the E or H (depending on polarization) side of the tube (Figure 1(b)). The specific locations are annotated on the figures, noting the results.

2. The effect of test-tube dimensions was determined using the data generated in the above exposures. Comparison of the small and large CN tubes for E, H, and K orientation is possible.

3. The effect of RFR frequency was determined by exposing the small CN test tube filled with muscle-equivalent material to 0.48, 1.2, 4.5, and 10 GHz with the tube oriented with its long axis parallel to the H-field only. SAR values were measured on the same axes as above.

4. The effect of the test-tube material was determined using the Styrofoam block of Figure 1 and only the small test-tube slot. Test tubes of the same outer diameter and length made of cellulose nitrate, polystyrene, and glass filled with muscle-equivalent material were exposed to RFR with the long dimension of the tube oriented parallel to the H-field. SAR values were measured on the same axes as above.

5. The effect of test-tube location within the RFR field (placement sensitivity for reproducible exposures of a single tube) was determined by moving a single CN test tube, oriented parallel to the H-field in the Styrofoam block of Figure 1, filled with muscle material, radially outward from the center of the RFR field. Movements toward and away from the horn were included as well. SAR values were measured only at the center of the test tube for each location.

6. Testing of the final design was performed using the Styrofoam holder shown in Figure 2. With tubes filled with muscle-equivalent material, the holder long axis oriented with test tubes parallel to the H-field and holder parallel with the E-field, SAR was measured in the center test tube at locations as above and at 2 cm into each of the 5 tubes to check for uniformity across the field.

RESULTS AND DISCUSSION

The effect of exposure orientation on the SAR in a single small and large CN test tube filled with muscle-equivalent material at 1.2 GHz is shown in Tables 2-3 and Figures 3-8. Both the small and large tubes exhibit similar orientation effects. It is clear from these data that the test tubes oriented with long axis parallel to the H-field have the most uniform distribution of absorbed energy.

The effect of test-tube size on SAR at 1.2 GHz for E, H, and K orientations can also be seen in the same tables and figures. The large CN tubes appear to absorb more RFR energy and have a more nonuniform energy distribution than the small CN tubes at 1.2 GHz. The approximate resonant frequencies of the small and large tubes were estimated at 2.5 and 1.9 GHz respectively (7). Since the large CN tube's resonant frequency is closer to the actual exposure frequency than the small tube, we would expect a higher rate of energy absorption and a more nonuniform absorption pattern. The results are, therefore, consistent with the theoretical predictions. It appears that the small and large CN test tubes oriented parallel with the H-field provide the most uniform SAR distribution, and this is therefore the preferred orientation for this exposure device. For H-field oriented test tubes the size does not appear to be a critical factor in the uniformity of the SAR distribution. The size in comparison to the frequency is the critical factor in the average SAR.

The effect of frequency on the SAR in small CN tubes oriented parallel with the H-field is shown in Tables 3 (center) and 4 and Figures 7 and 9-11 for 0.48, 1.2, 4.5, and 10.0 GHz. The energy distributions within the tubes at all frequencies are fairly uniform but the average SARs differ in magnitude significantly at the different exposure frequencies. The average SARs for the tubes are 0.0187, 0.064, 0.677, and 0.622 W/kg per mW/cm^2 respectively. A comparison of these data to predicted SAR values in a prolate spheroid of comparable size and shape is shown in Figure 12 (7). The prolate spheroid chosen for comparison is not in exact proportion to the test tube used but does provide a valid comparison of the resonant frequency and average SAR. The experimental values appear to correspond quite well with the theoretical values showing that SAR is a function of frequency with low SAR at frequencies well below resonance and high values at and above resonance.

The effect of test-tube material on the SAR distribution in H-oriented small test tubes at 1.2 GHz can be seen in Table 5 and Figures 7, 13, and 14 for the CN, glass, and polystyrene tubes, respectively. It appears that the SAR distribution is more uniform, and the average SAR higher in the CN tubes than in either glass or polystyrene. This would indicate that the CN tubes provide less interaction with the RF field and are of the preferable material to use for the exposure device.

The effect of H-oriented CN test-tube location in the RFR field at 1.2 GHz is shown in Table 6. Moving across the field parallel to the E-field has no effect until exceeding 10 cm off the central axis, indicating that the RF field was fairly uniform out to 10 cm. Moving the tube away from the horn shows a decrease in SAR, indicating the expected decrease in the field intensity. Moving toward the horn also shows a decrease in SAR, which might not have been expected except that calculations show the far field of the horn starts at approximately 0.8 m from the horn. Moving the tubes toward the horn would cause a decrease in the SAR because of the nonuniformity of the E and H fields in the near field. These experiments indicate that the location of the test tube in the RFR field is critical with respect to the distance from the horn but is fairly insensitive to distance off centerline as long as the uniform field size is large compared to the object being irradiated.

The testing of the final design of the in vitro sample holder is shown in Table 7. The SAR distribution in the centrally located test tube appears to be the same as the single test tube exposed alone. In addition, the SAR at 2 cm into each tube appears approximately the same for each tube, indicating that arrangement of the tubes across the E-field axis has little scatter effect. It appears that the exposure of 5 test tubes at the same time in the configuration shown in Figure 2 provides uniform exposures of all tubes.

CONCLUSIONS

The results of these experiments have shown that it is possible to design a simple, low-cost, in vitro exposure device that will provide known, uniform, and reproducible RFR exposures. These experiments have shown that cellulose nitrate is the preferable material of construction over glass or polystyrene, that orientation of the test tubes with long axis parallel with the RFR H-field provides the most uniform exposures throughout the frequency range 0.48 to 10 GHz, that exposure frequency vs. the size of the test tube significantly affects the SAR with low values of SAR well below resonance and high values of SAR at and above resonance, that CN tubes in an array parallel to the E-field can be uniformly exposed in gang fashion and still provide consistent exposures of each tube, and that the location of the test-tube array in the RFR field is critical for distance from the horn but fairly insensitive across the axis of the horn.

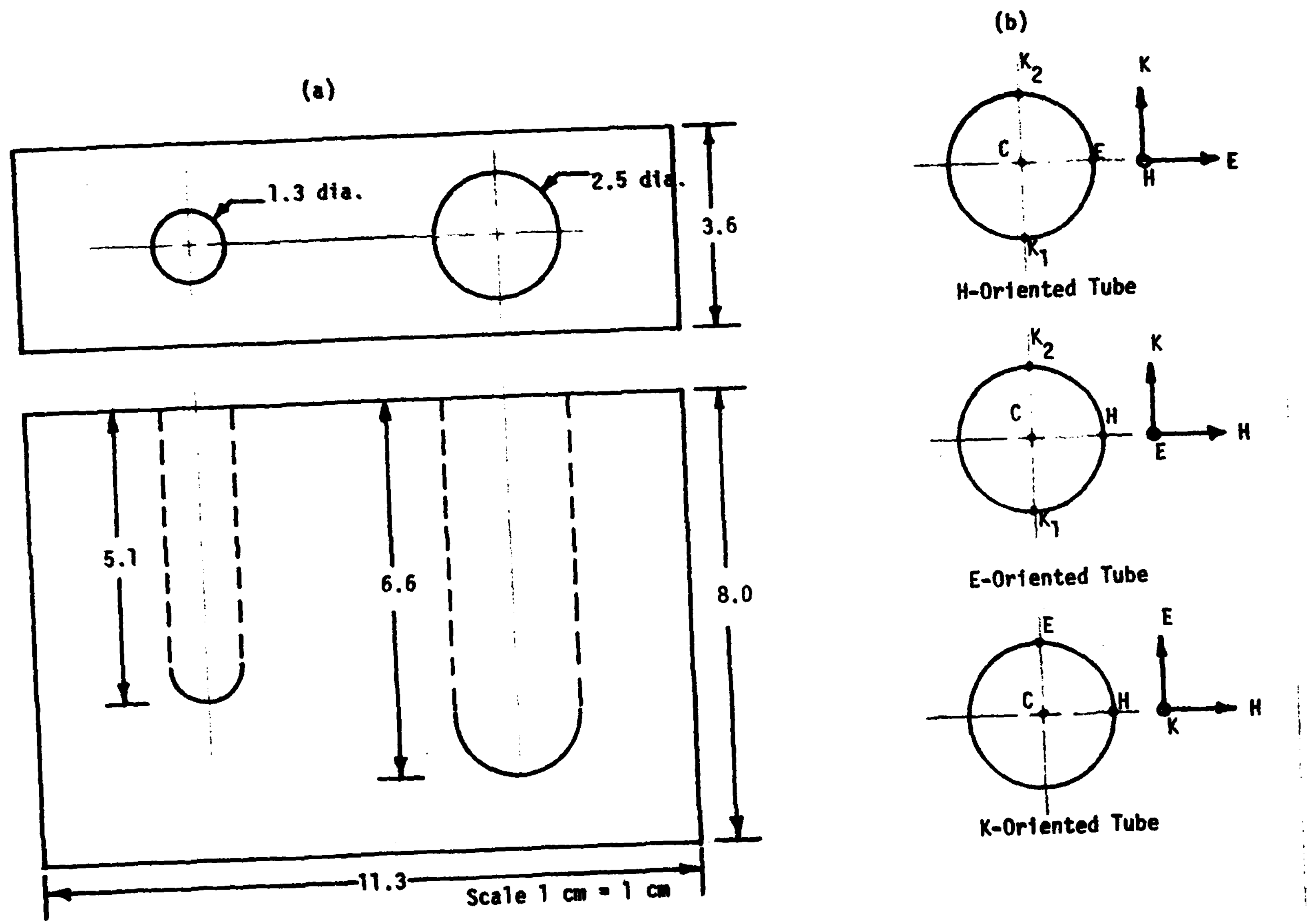

Figure 1(a). Styrofoam test-tube holder for determining effect of exposure orientation, effect of frequency, effect of test-tube size, effect of test-tube material, and effect of test-tube location in RFR field.

(b). Orientation of temperature-probe axes E, H, K_1, and K_2, and C (center) for tubes oriented parallel to E, H, and K.

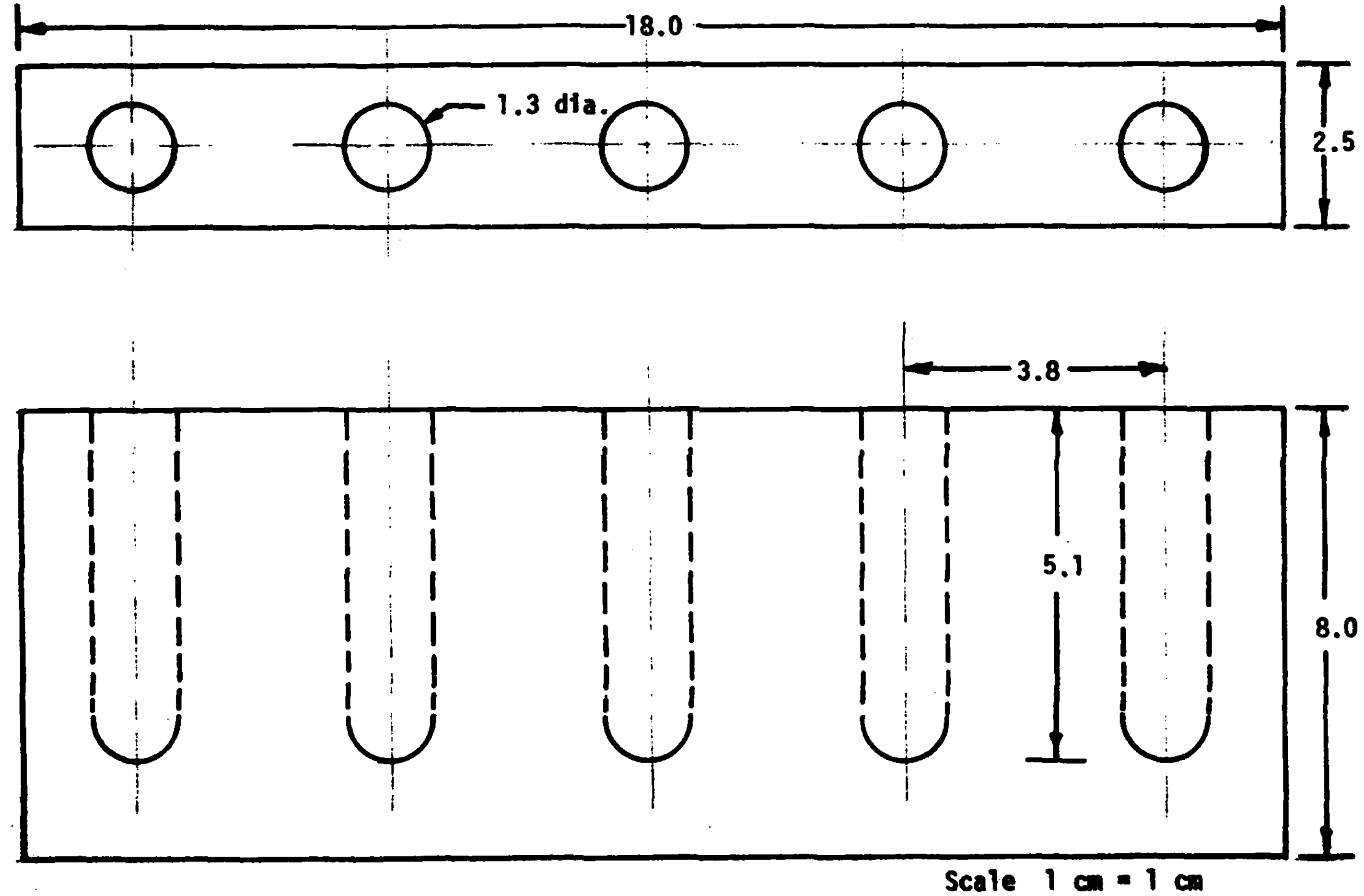

Figure 2. Final design of Styrofoam in vitro sample holder containing 5 cellulose-nitrate tubes.

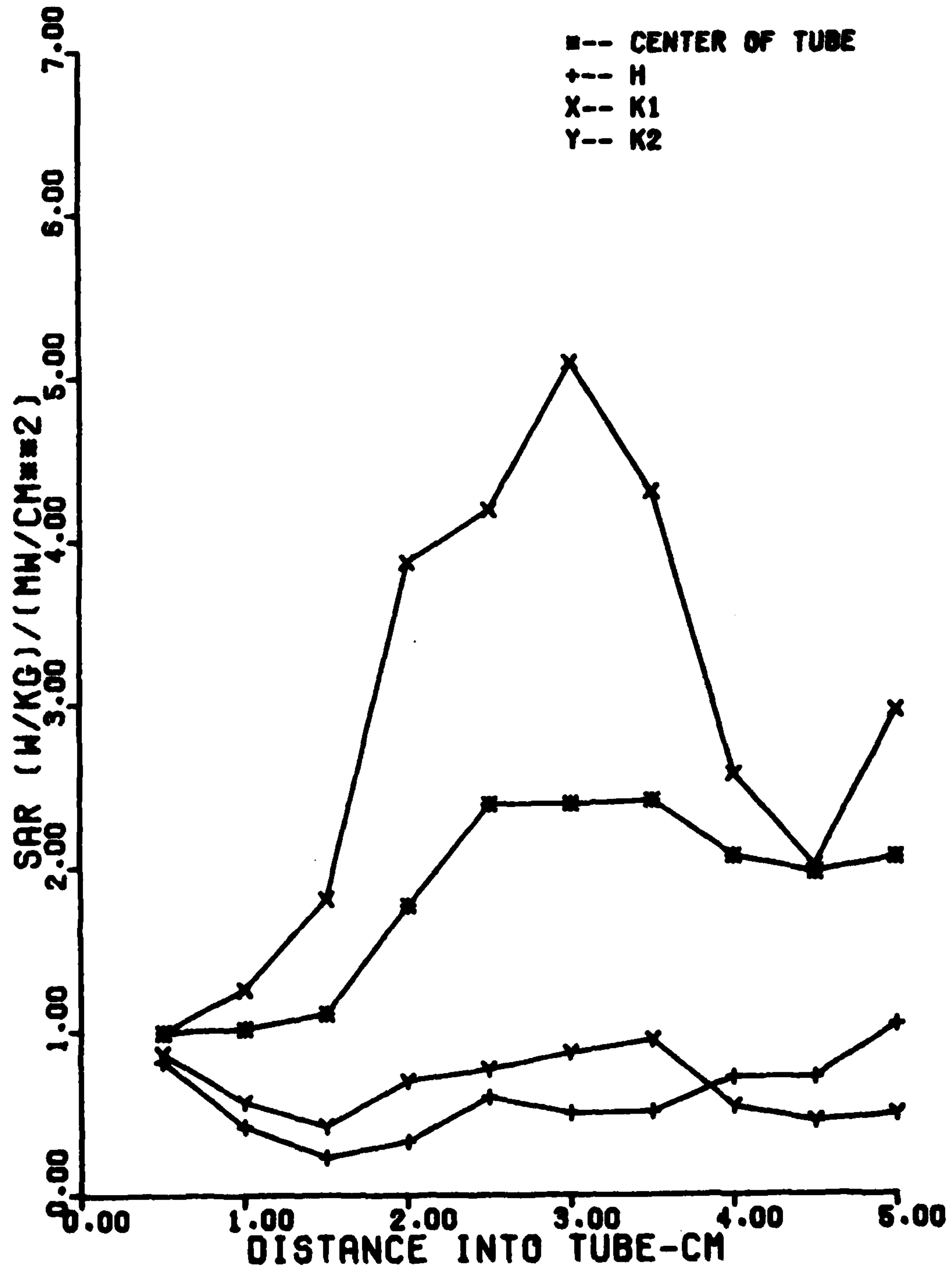

Figure 3. SAR in E-oriented 2.5- x 6.6-cm CN tube filled with muscle-equivalent material exposed to 1.2 GHz, continuous wave (CW), far field, 70 mW/cm^2.

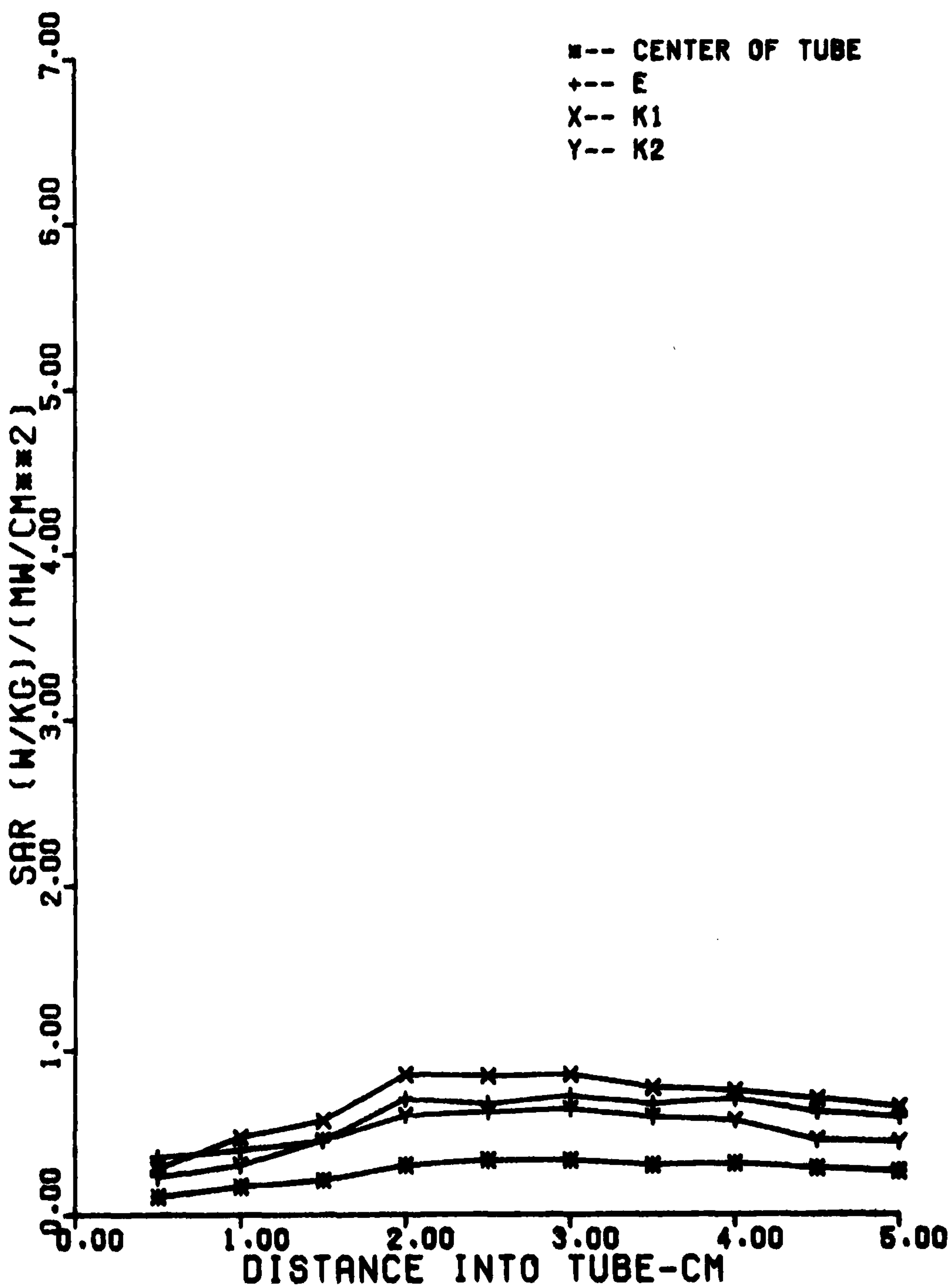

Figure 4. SAR in H-oriented tube as in Figure 3.

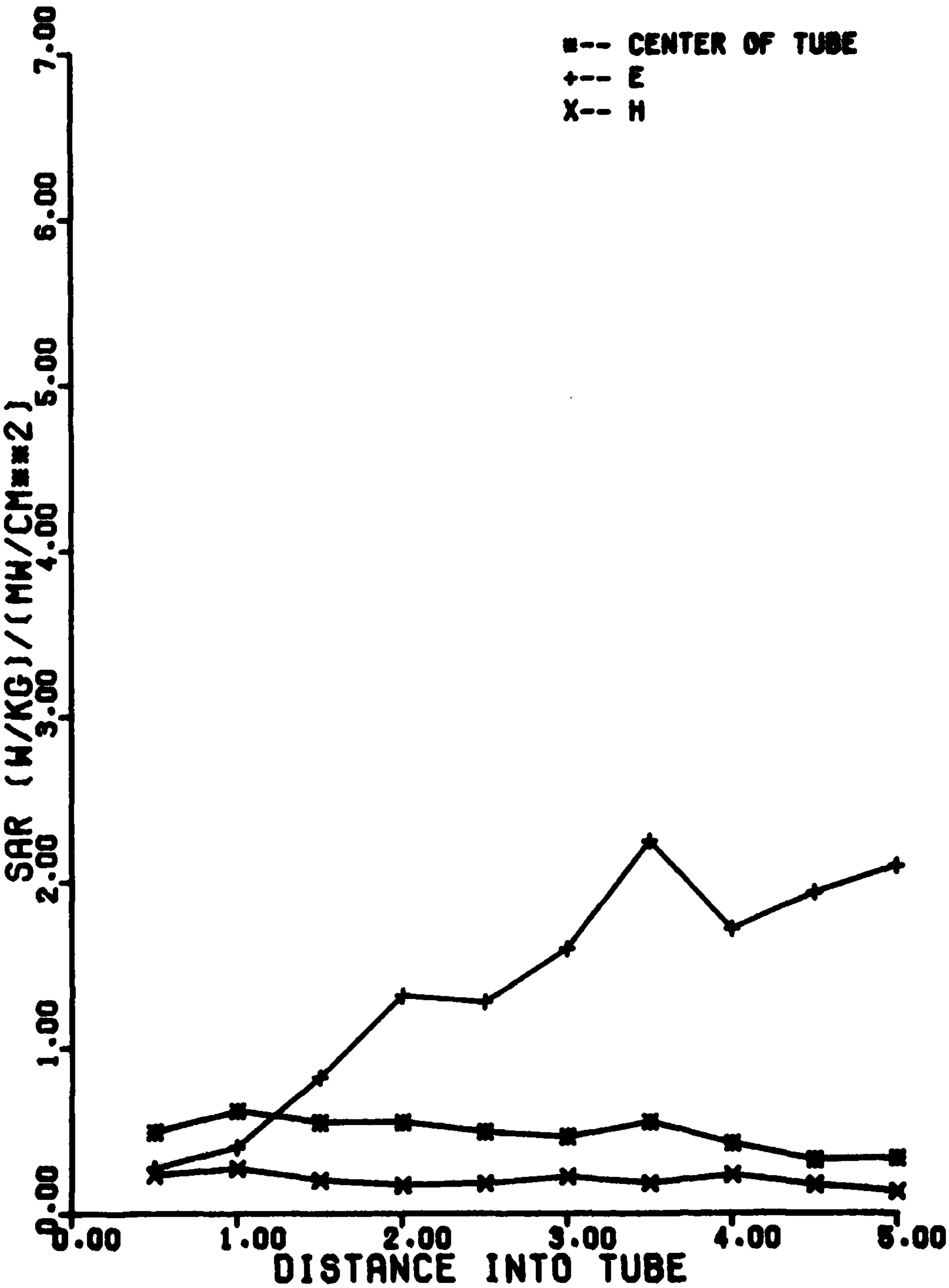

Figure 5. SAR in K-oriented tube as in Figure 3.

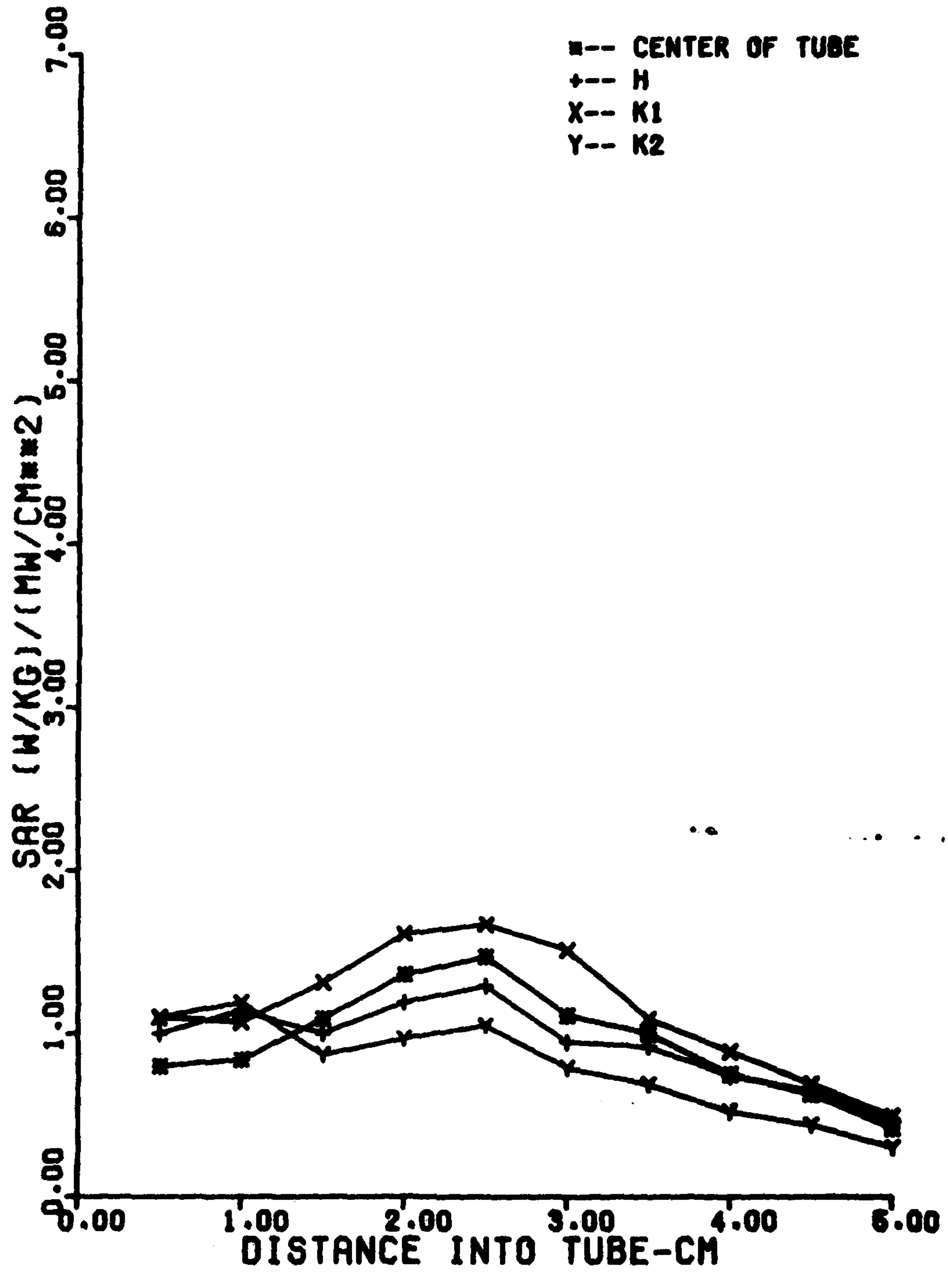

Figure 6. SAR in E-oriented 1.3- x 5.1-cm CN tube filled with muscle-equivalent material exposed to 1.2 GHz, CW, far field, 70 mW/cm^2.

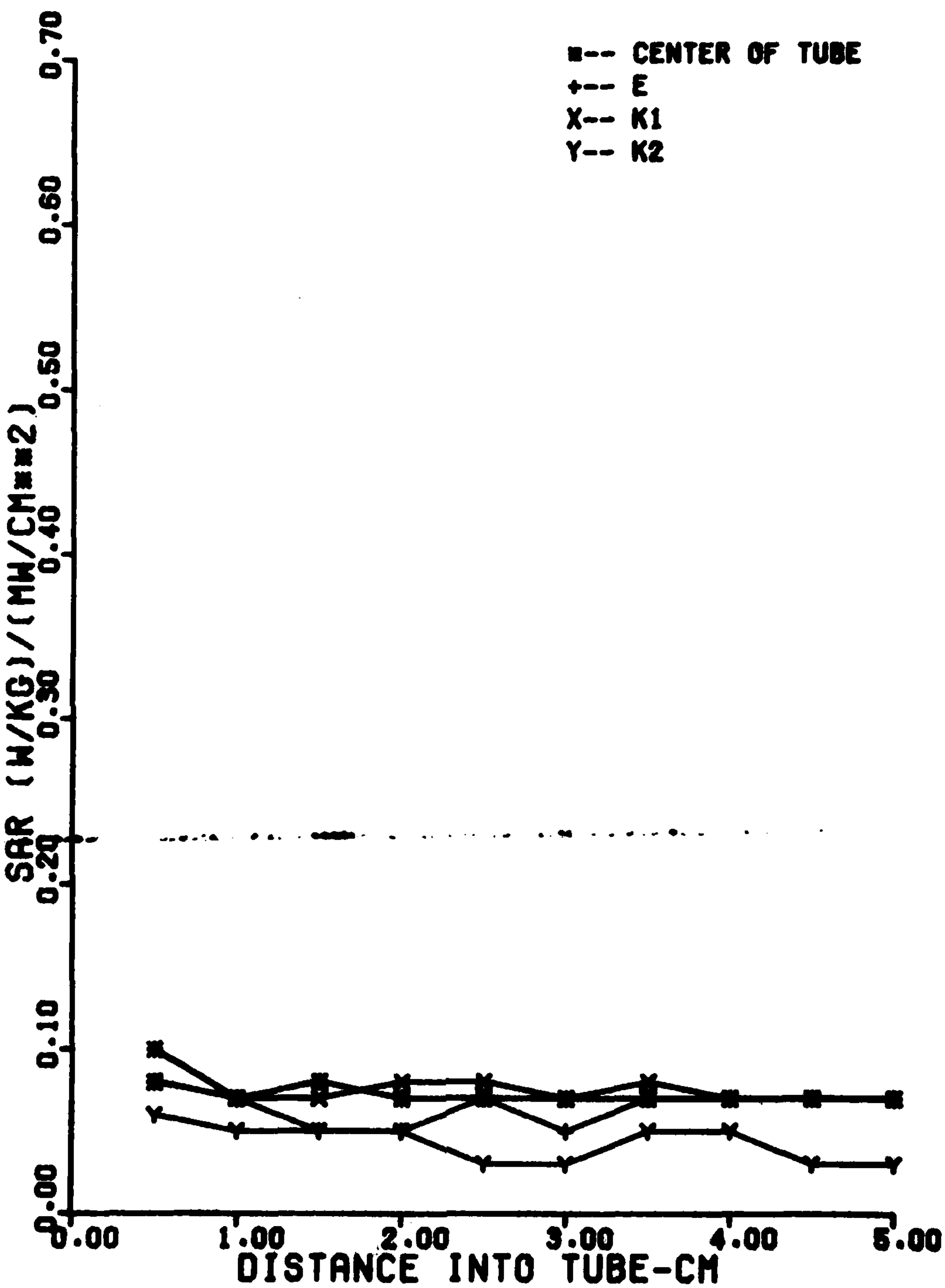

Figure 7. SAR in H-oriented tube as in Figure 6.

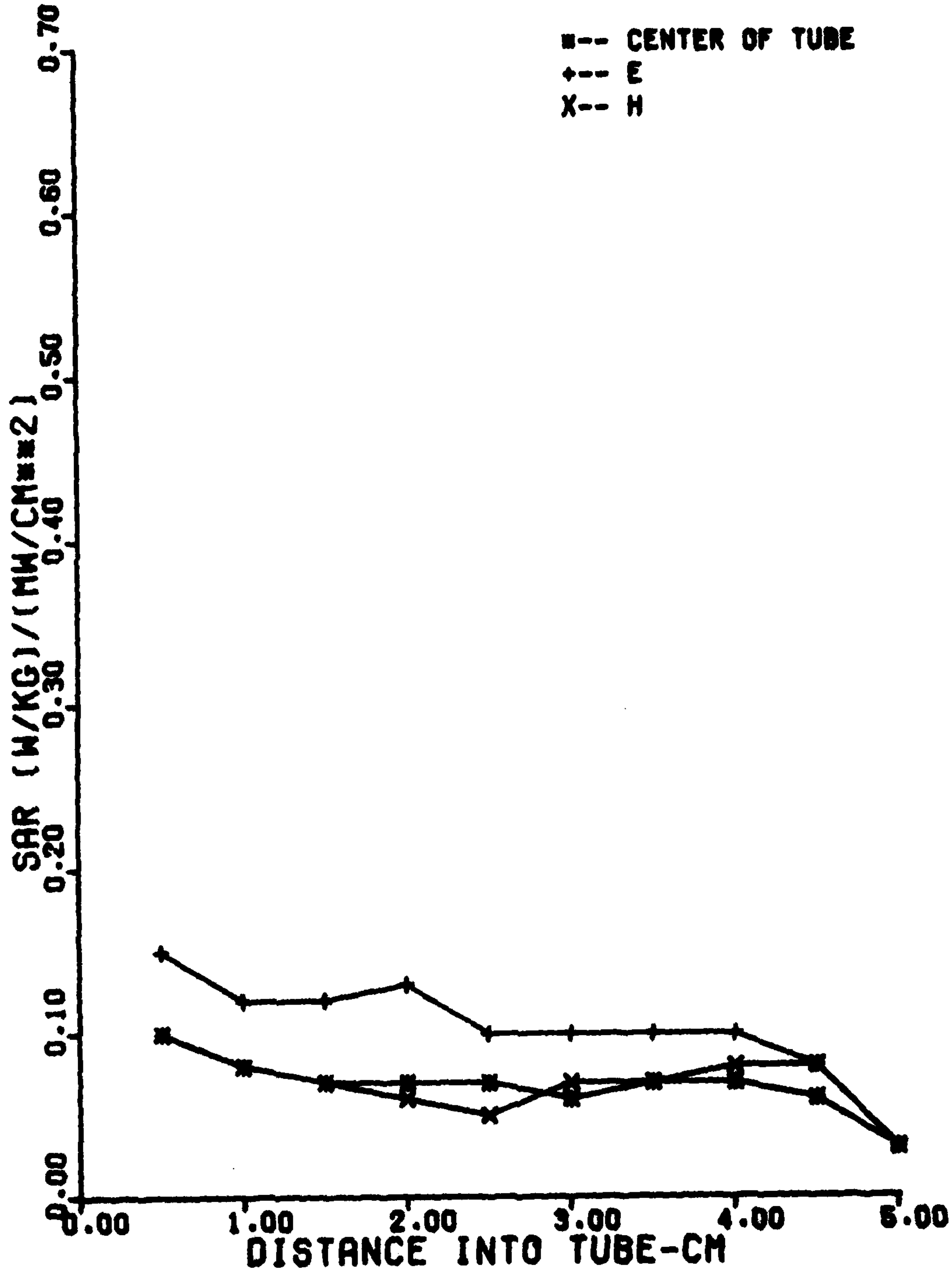

Figure 8. SAR in K-oriented tube as in Figure 6.

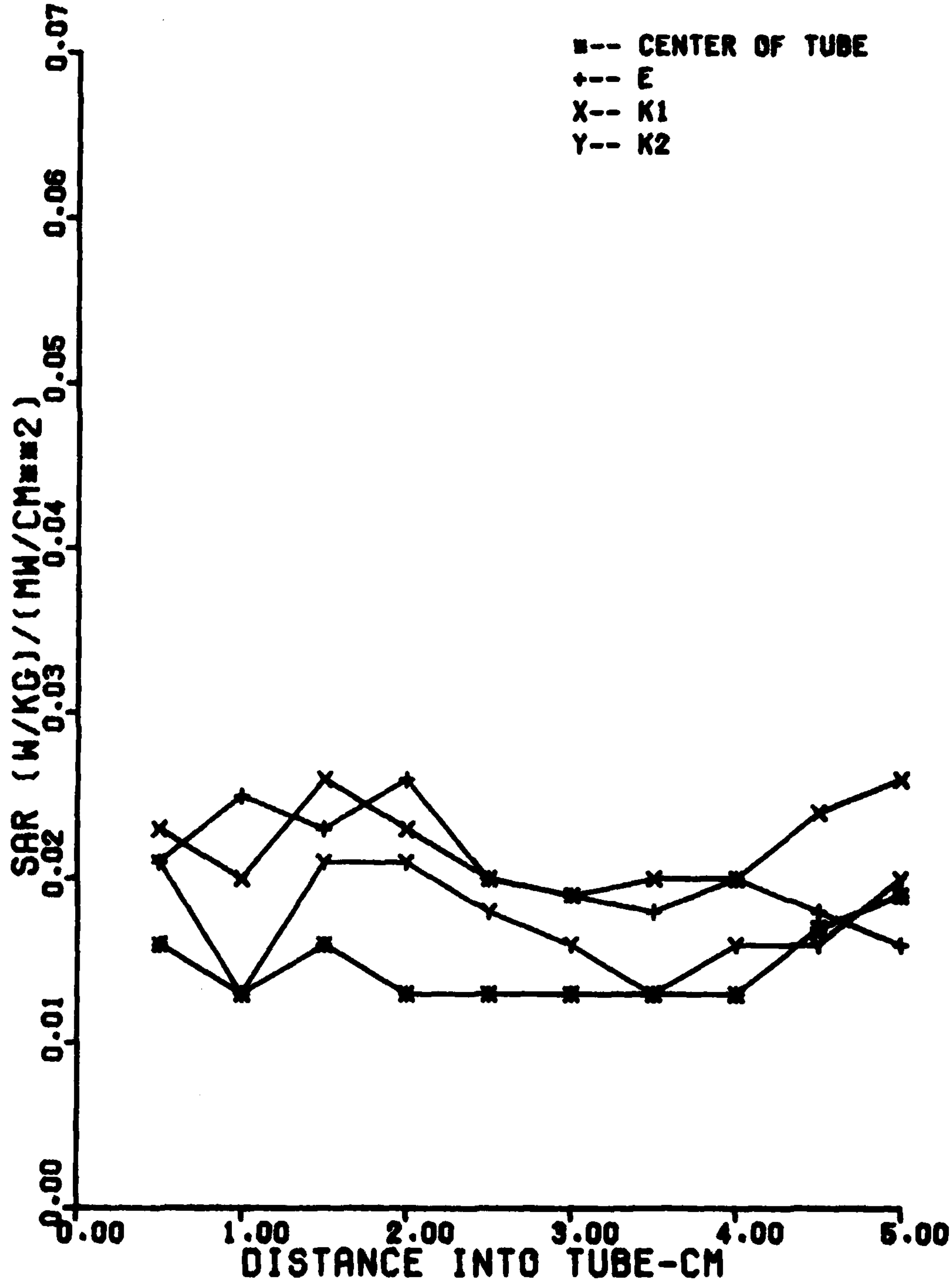

Figure 9. SAR in H-oriented 1.3- x 5.1-cm CN tube filled with muscle-equivalent material exposed to 0.48 GHz, continuous wave (CW), far field, 450 mW/cm^2.

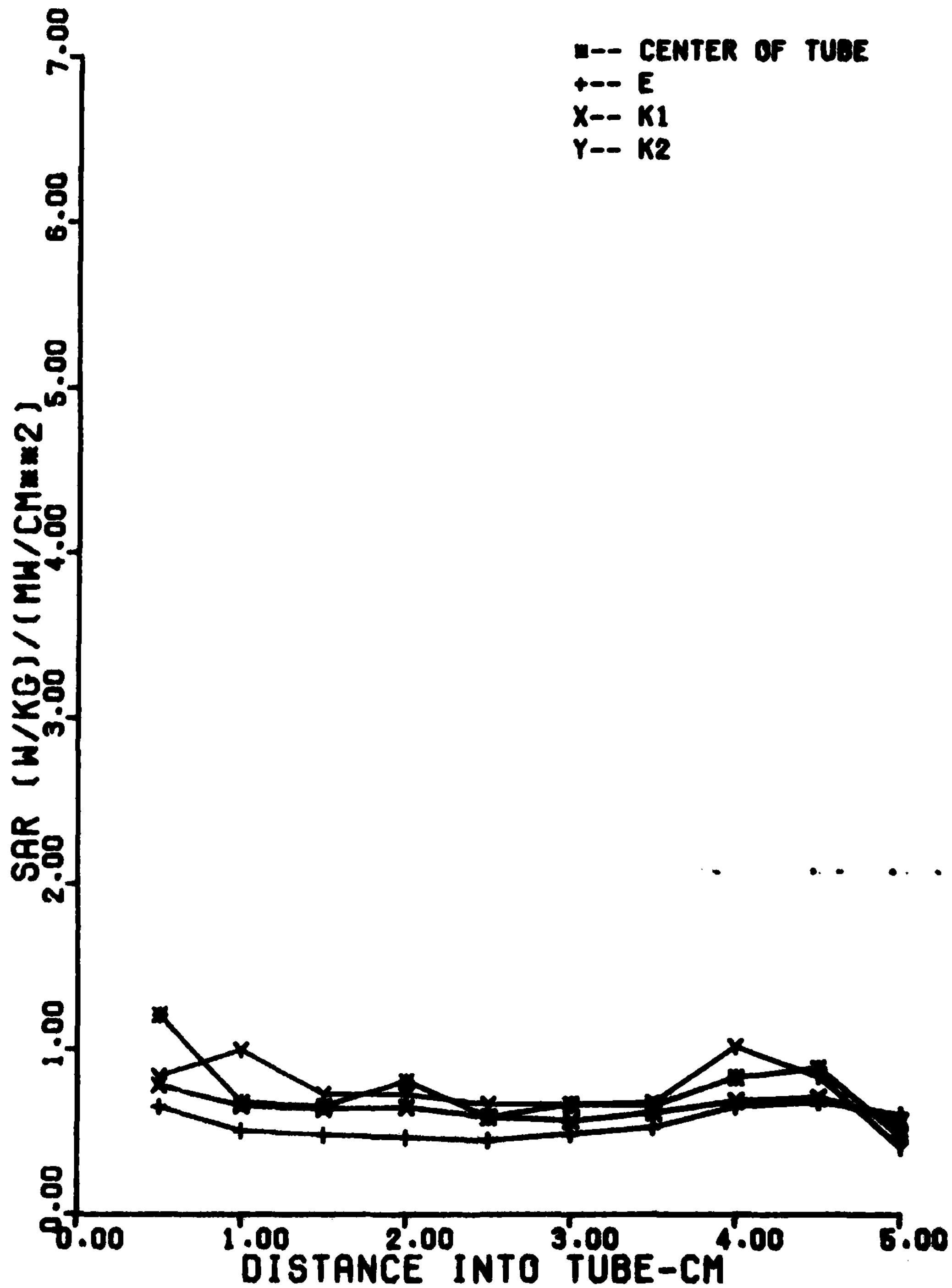

Figure 10. SAR in H-oriented 1.3- x 5.1-cm CN Tube filled with muscle-equivalent material exposed to 4.5 GHz, CW, far field, 100 mW/cm^2.

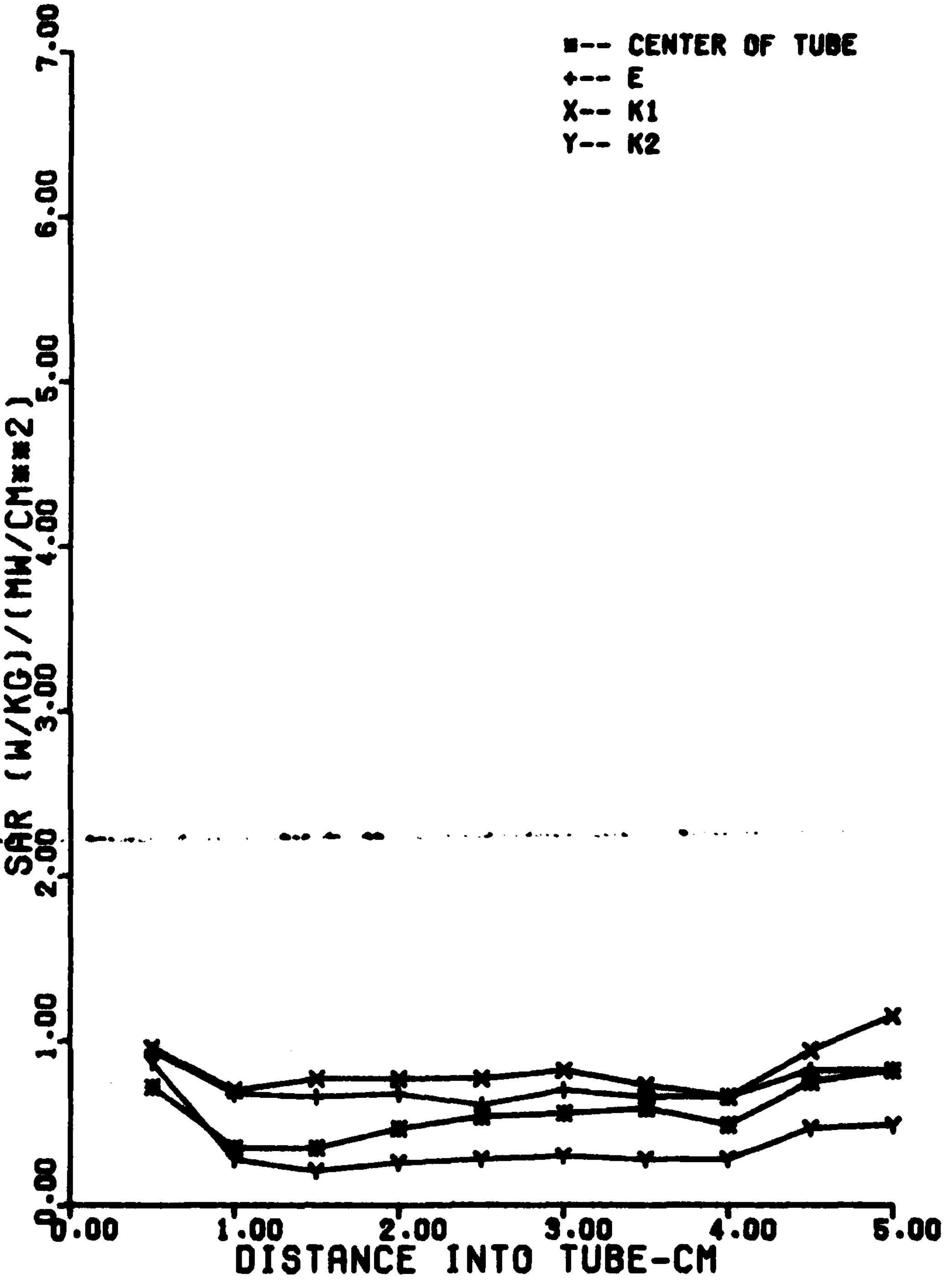

Figure 11. SAR in H-oriented 1.3- x 5.1-cm CN tube filled with muscle-equivalent material exposed to 10.0 GHz, CW, far field, 50 mW/cm^2.

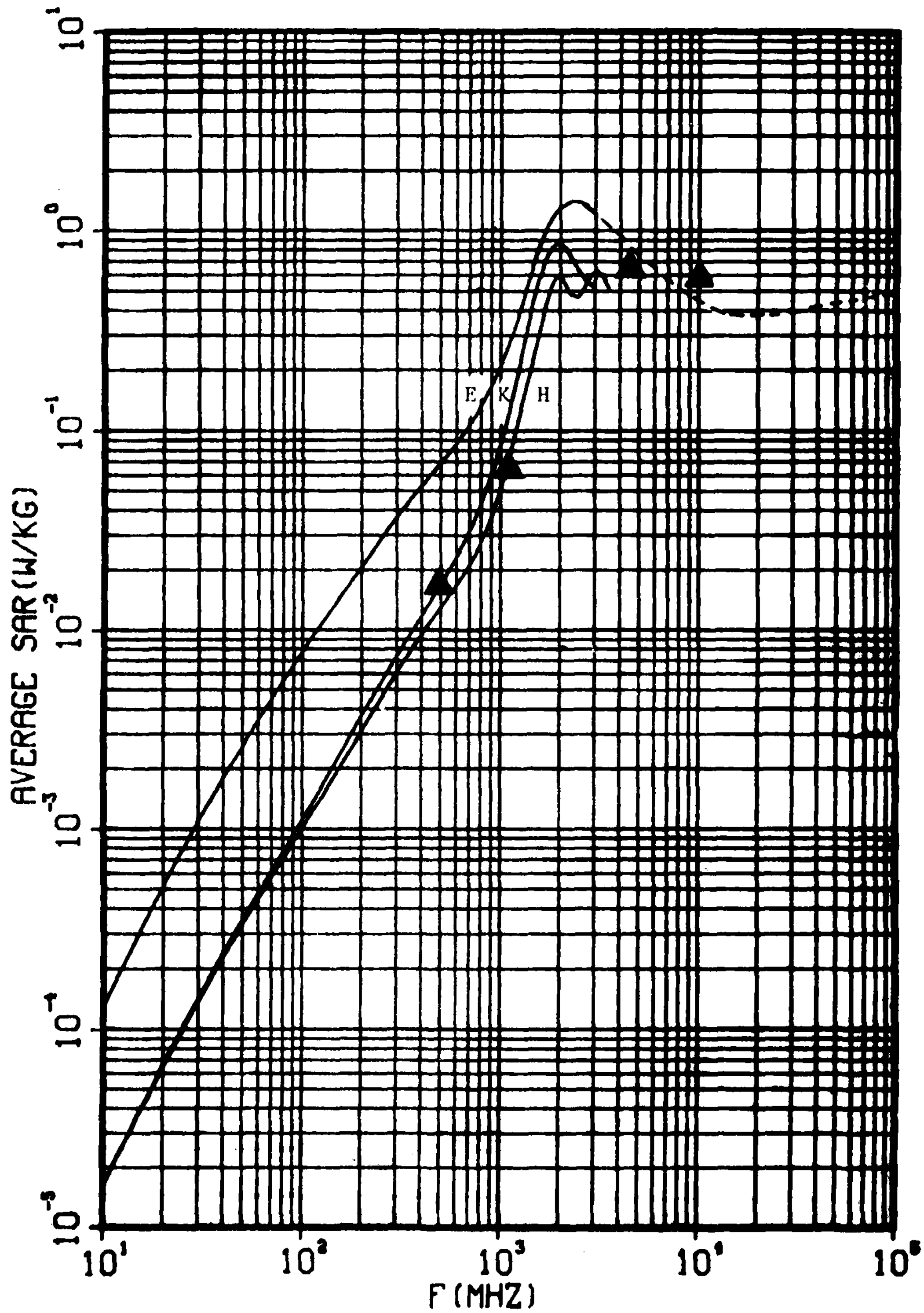

Figure 12. Average SAR vs. frequency for the 0.48, 1.2, 4.5, and 10 GHz exposures in 1.3- x 5.1-cm CN tubes compared to average SAR in a prolate spheroid of dimensions 2.3 cm wide and 5.4 cm long.

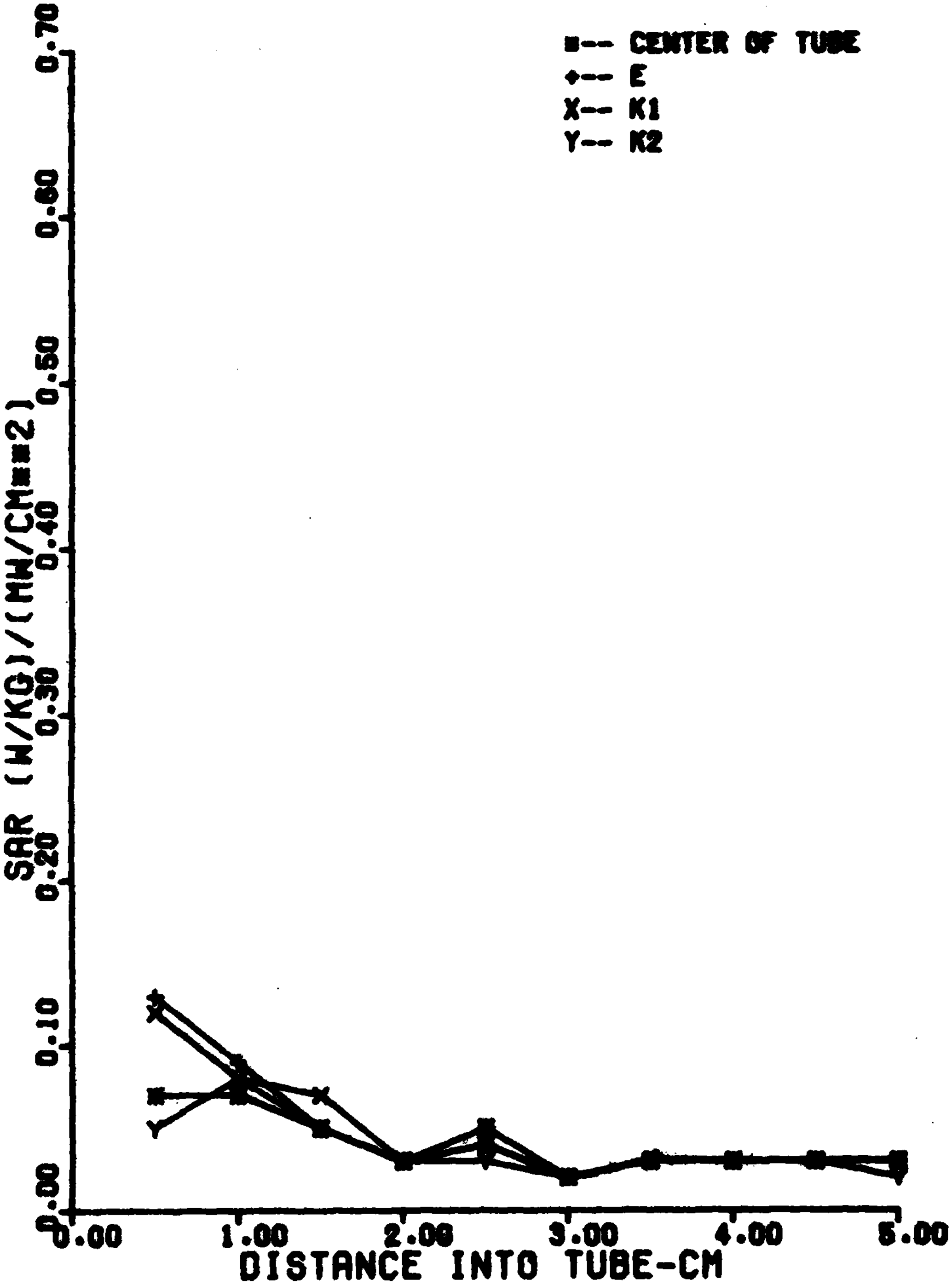

Figure 13. SAR in H-oriented 1.3- x 5.1-cm glass test tube filled with muscle-equivalent material exposed to 1.2 GHz, CW, far field, 70 mW/cm^2.

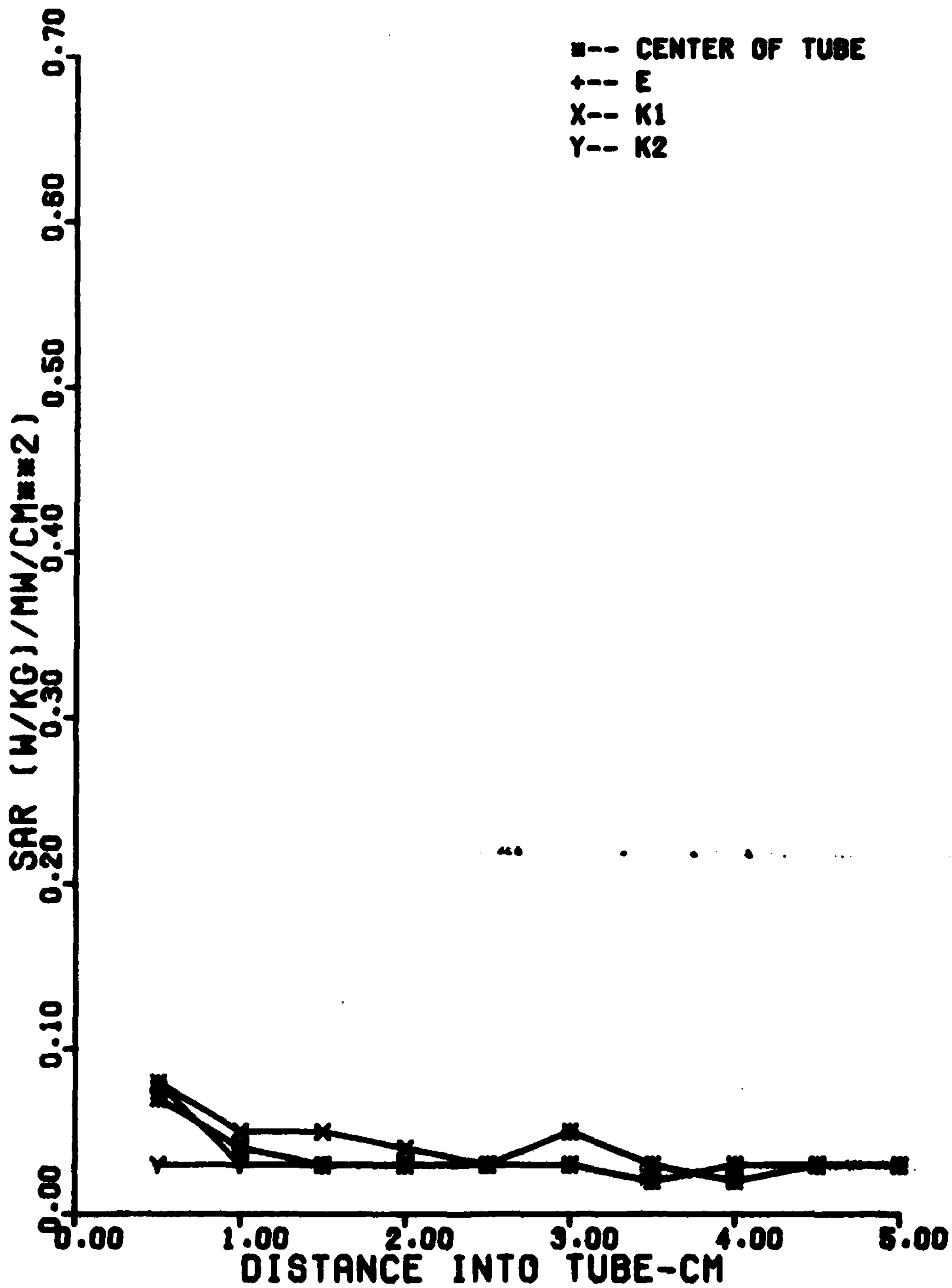

Figure 14. SAR in H-oriented 1.3- x 5.1-cm polystyrene test tube filled with muscle-equivalent material exposed to 1.2 GHz, CW, far field, 70 mW/cm^2.

TABLE 2. EFFECT OF EXPOSURE ORIENTATION FOR LARGE CN TUBE

	Parallel to E-vector				Parallel to H-vector				Parallel to K-vector		
Depth (cm)	Center	K_1	H	K_2	Center	K_1	E	K_2	Center	E	H
0.50	1.00	1.00	0.82	0.87	0.11	0.78	0.35	0.23	0.49	0.27	0.23
1.00	1.02	1.26	0.42	0.57	0.17	0.47	0.39	0.30	0.62	0.40	0.27
1.50	1.11	1.81	0.23	0.42	0.21	0.57	0.45	0.45	0.55	0.82	0.20
2.00	1.77	3.87	0.33	0.70	0.30	0.85	0.70	0.60	0.55	1.31	0.17
2.50	2.39	4.19	0.60	0.77	0.33	0.84	0.67	0.62	0.49	1.27	0.18
3.00	2.39	5.09	0.50	0.87	0.33	0.85	0.72	0.64	0.46	1.59	0.22
3.50	2.41	4.30	0.51	0.95	0.30	0.77	0.67	0.59	0.55	2.24	0.18
4.00	2.07	2.57	0.72	0.54	0.31	0.75	0.70	0.57	0.42	1.71	0.23
4.50	1.97	2.01	0.77	0.45	0.28	0.70	0.62	0.45	0.32	1.93	0.17
5.00	2.06	2.96	1.04	0.49	0.26	0.65	0.59	0.44	0.33	2.09	0.13

1.2 GHz, CW, 0.81 m from horn, 70 mW/cm^2; 2.5- x 6.6-cm cellulose nitrate test tube filled with muscle-equivalent material. SAR in $\frac{W/kg}{mW/cm^2}$

TABLE 3. EFFECT OF EXPOSURE ORIENTATION FOR SMALL CN TUBE

	Parallel to E-vector				Parallel to H-vector				Parallel to K-vector		
Depth (cm)	Center	K_1	H	K_2	Center	K_1	E	K_2	Center	E	H
0.50	0.80	1.00	1.10	1.10	0.08	0.10	0.10	0.06	0.10	0.15	0.10
1.00	0.84	1.07	1.14	1.19	0.07	0.07	0.07	0.05	0.08	0.12	0.08
1.50	1.09	1.31	1.00	0.87	0.07	0.07	0.05	0.05	0.07	0.13	0.07
2.00	1.36	1.61	1.19	0.07	0.07	0.08	0.05	0.05	0.07	0.10	0.06
2.50	1.47	1.67	1.29	1.05	0.07	0.08	0.07	0.03	0.06	0.10	0.05
3.00	1.11	1.51	0.95	0.79	0.07	0.07	0.05	0.03	0.07	0.10	0.07
3.50	1.00	1.09	0.92	0.69	0.07	0.08	0.07	0.05	0.07	0.10	0.07
4.00	0.75	0.89	0.74	0.52	0.07	0.07	0.07	0.05	0.07	0.10	0.08
4.50	0.63	0.69	0.65	0.44	0.07	0.07	0.07	0.03	0.06	0.08	0.08
5.00	0.42	0.49	0.47	0.30	0.07	0.07	0.07	0.03	0.03	0.03	0.03

1.2 GHz, CW, 0.81 m from horn, 70 mW/cm^2; 1.3- x 5.1-cm cellulose-nitrate test tube filled with muscle-equivalent material. SAR in $\frac{W/kg}{mW/cm^2}$

TABLE 4. EFFECT OF FREQUENCY

	0.48 GHz (450 mW/cm^2)				4.5 GHz (100 mW/cm^2)				10 GHz (50 mW/cm^2)			
Depth (cm)	Center	K_1	E	K_2	Center	K_1	E	K_2	Center	K_1	E	K_2
0.50	0.016	0.023	0.021	0.021	1.21	0.79	0.66	0.84	0.72	0.96	0.94	0.87
1.00	0.013	0.020	0.025	0.013	0.68	0.66	0.51	1.01	0.35	0.70	0.68	0.28
1.50	0.016	0.026	0.023	0.021	0.66	0.64	0.49	0.73	0.35	0.77	0.66	0.21
2.00	0.013	0.023	0.026	0.021	0.81	0.65	0.47	0.73	0.47	0.77	0.68	0.26
2.50	0.013	0.020	0.020	0.018	0.60	0.59	0.45	0.67	0.54	0.77	0.61	0.28
3.00	0.013	0.019	0.019	0.016	0.67	0.57	0.49	0.67	0.56	0.82	0.70	0.30
3.50	0.013	0.020	0.018	0.013	0.63	0.62	0.53	0.68	0.59	0.73	0.66	0.28
4.00	0.013	0.020	0.020	0.016	0.83	0.69	0.66	1.02	0.49	0.66	0.66	0.28
4.50	0.017	0.024	0.018	0.016	0.89	0.71	0.69	0.84	0.75	0.94	0.82	0.47
5.00	0.019	0.026	0.016	0.020	0.48	0.57	0.61	0.41	0.82	1.15	0.82	0.49

All exposures CW, H-oriented; 1.3- x 5.1-cm cellulose-nitrate test tube filled with homogeneous muscle-equivalent material. SAR in $\frac{W/kg}{mW/cm^2}$

TABLE 5. EFFECT OF TEST-TUBE MATERIAL

	Polystyrene				Glass				Cellulose Nitrate			
Depth (cm)	Center	K_1	E	K_2	Center	K_1	E	K_2	Center	K_1	E	K_2
0.50	0.07	0.08	0.08	0.03	0.07	0.12	0.13	0.05	0.08	0.10	0.10	0.06
1.00	0.04	0.05	0.03	0.03	0.07	0.08	0.09	0.08	0.07	0.07	0.07	0.05
1.50	0.03	0.05	0.03	0.03	0.05	0.07	0.05	0.05	0.07	0.07	0.05	0.05
2.00	0.03	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.07	0.08	0.05	0.05
2.50	0.03	0.03	0.03	0.03	0.05	0.04	0.04	0.03	0.07	0.08	0.07	0.03
3.00	0.03	0.05	0.05	0.03	0.02	0.02	0.02	0.02	0.07	0.07	0.05	0.03
3.50	0.02	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.07	0.08	0.07	0.05
4.00	0.03	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.07	0.07	0.07	0.05
4.50	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.07	0.07	0.07	0.03
5.00	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.07	0.07	0.07	0.03

All exposures at 1.2 GHz, H-oriented, 70 mW/cm^2, 1.0 meters from horn; 1.3- x 5.1-cm test tubes filled with homogeneous muscle-equivalent material. SAR in $\frac{W/kg}{mW/cm^2}$

TABLE 6. EFFECT OF TEST-TUBE LOCATION

Exposure Location	SAR (W/kg)
Centered	4.69
5 cm left	4.69
10 cm left	4.69
15 cm left	4.10
20 cm left	3.52
25 cm left	3.52
Center (repeat)	4.69
5 cm Towards Horn	4.10
10 cm Towards Horn	3.52
5 cm Away from Horn	3.52

TABLE 7. EFFECT OF TEST-TUBE ARRAY

SAR Distribution in Central Tube					SAR in other Tubes			
Depth (cm)	Center	K_1	E	K_2	Tube 1	Tube 2	Tube 4	Tube 5
0.5	0.067	0.067	0.150	0.050				
1.0	0.084	0.050	0.084	0.050				
1.5	0.084	0.084	0.084	0.050				
2.0	0.067	0.067	0.084	0.033	0.084	0.067	0.059	0.067
2.5	0.084	0.050	0.067	0.033				
3.0	0.084	0.067	0.084	0.050				
3.5	0.067	0.067	0.084	0.033				
4.0	0.067	0.050	0.067	0.033				
4.5	0.084	0.067	0.067	0.033				
5.0	0.084	0.067	0.067	0.033				

1.2 GHz, CW, 70 mW/cm^2, 1.0 m from horn; 1.3- x 5.1-cm cellulose-nitrate test tubes arranged as shown in Figure 2. SAR in $\frac{W/kg}{mW/cm^2}$